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LabLink

this issue

Director,
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Fungal Identification To Resume

Starting Oct. 1, 2014-Identification of filamentous fungi from all specimen sites

Beginning October 1, 2014, the Michigan Department of Community Health (MDCH) Bureau of Laboratories (BOL) mycology lab will once again accept referred isolates for fungal identification of filamentous fungi from all specimen sources. We have extensive experience in fungal identification and we are pleased to resume this service for you. Yeast and dermatophyte identification are not acceptable for identification at this time. Questions about this service may be phoned to 517-335-9637.

CAP ACCREDITED

Michigan Department of Community Health
MDCH
Rick Snyder, Governor
James K. Haveman, Director

Job Posting: Microbiology Section Manager – State Administrative Manager 15

Position Description: The Microbiology Section Manager serves as the administrator and technical director of the laboratory that provides diagnostic and reference testing for bacterial agents of public health importance such as *M. tuberculosis*, the agents of bioterrorism, agents associated with foodborne illnesses, and antibiotic resistant organisms. The Section participates in the Laboratory Response Network, the Food Emergency Response Network, serves as the mid-west PulseNet area lab, and provides TB genotyping services for TB control programs in the United States. The position plans and directs the activities of the Microbiology Section (highly complex scope) through first-line supervisors. This position defines work objectives, establishes priorities for the Section and directs investigative work to develop improved testing services and new methodologies.

Preferred Education: Doctoral degree in Microbiology, Virology, Laboratory Practice, Clinical Sciences, Hospital and Molecular Epidemiology.

Salary: 69,707.00 - $100,267.00

For further information, please contact James T. Rudrik, Ph.D., Director, Infectious Disease Division at 517-335-8067 or rudrikj@michigan.gov

Enterovirus-D68 - Virus with an Identity Crisis

Janice Matthews-Greer, Ph.D., D(ABMM)

On September 8, 2014, the Centers for Disease Control and Prevention announced it is investigating clusters of sick children hospitalized with severe respiratory infections in Kansas City, MO and Chicago, IL. The contagion as confirmed in more than 80% of the patients sampled is the human-Enterovirus-D68. The majority of the sick children had a previous diagnosis of asthma, wheezing or some type of reactive airway disease. Now it appears the virus has spread with clusters in at least a dozen states. Enteroviruses cause millions of infections in the United States every year. They belong to the family Picornaviridae ("small" "rna" virus), a large growing family of at least 17 genera. These are very simple both structurally and genetically. The genome ("chromosome") is referred to as positive RNA because like cellular mRNA (messenger RNA), it can be immediately translated into protein – in this case viral protein which enables it to kill the cell. Picornaviruses are perhaps best known by older terms designating groups: polioviruses, Coxsackie A (of which A6 is currently causing an outbreak of Hand, Foot and Mouth Disease (HFMD), Coxsackie B, viruses and the ECHOs (Enteric Cytopathic Human Orphan viruses)). Newer members are given numbers and assigned to a lettered species, e.g., EV-D68, all due to new molecular classification techniques. Likewise the old groups have been re-classified into lettered species. It is now possible for an enterovirus to be discussed between a young and (Ahem...) "older" virologist with neither one knowing which agent is on topic.

There are at least 12 species and more than 100 strains of human viruses within the *Enterovirus* genus. Within the genus are the rhinoviruses, most of which are guilty of causing infections limited to the upper respiratory tract such as the "common cold and sinusitis". In species EV-D are several types of enteroviruses, which cause a variety of illnesses, but most commonly the "summer flu" or "summer cold" in late summer, early fall. As a rule enteroviruses differ from rhinoviruses in their ability to replicate in the gut rather than the nasal epithelium, hence fecal-oral spread in addition to droplet transmission, their resistance to low pH, their higher optimal growth temperature, and the viremic spread from the lymph nodes to other body sites and organs. Thus, they are capable of causing both upper and lower tract respiratory infection with possible inflammation of the meninges (aseptic meningitis) and even encephalitis. Other enteroviruses (non-D) are common causes of myelitis and inflammation of the spinal cord (flaccid paralysis), the heart muscle, the liver and the pancreas. Some are known for causing hand-foot-and-mouth-disease (currently Coxsackie A6 is circulating) and conjunctivitis. And although replication occurs in the gut, gastroenteritis is not the usual presentation. ►

The likely culprit of the Michigan outbreak of respiratory disease in children, like the rest of the Mid-West, EV-D68 is unique for several reasons. First of all, in that rhinoviruses and enteroviruses share genetic homology and similar structure, strains of EV-D68 have previously been classified as a rhinovirus (RV-87). Like rhinoviruses, Enterovirus-D68 grows optimally at 33°C and because it is sensitive to stomach acid, can test negative with fecal samples or swabs. Unlike a rhinovirus however, EV-D68 is capable of causing severe lower respiratory and neurologic disease.

EV-D68 was first described in California in 1962 in 3 children with severe respiratory illness and 1 infant with pneumonia. It then either dropped from sight, rarely reported as an agent of respiratory disease in hospitalized children, or it was classified as a rhinovirus based upon growth temperature and acid liability tests. But for some unknown reason, the D-68 strain of Enterovirus has risen with a vengeance and appears to have mounted a major attack in recent years, first in Asia and Europe and now (since 2009) in the United States. Neutralizing antibody present in archived sera prove EV-D68 did circulate during this period of perceived inactivity. No doubt numerous cases are asymptomatic or mild. However, illness can be extremely severe, presenting as hypoxia or asthma exacerbation, primarily in children with comorbidities. Seizures and neurologic abnormalities are noted in some, intensive care for many and even extracorporeal membrane oxygenation (ECMO). Fatalities are not common. Most patients requiring hospitalization are pediatric, but asymptomatic infection in adults and severe respiratory illness in the elderly are under investigation as well.

Our current outbreaks in the mid-western part of the United States do seem to have a higher morbidity rate than previously seen. One school of thought as to the reason for increased virulence is due to deletions found in the genome of this current circulating clade. The deletions enhance the translational efficiency (the virus's ability to make protein), thereby enhancing viral fitness over previous clades. In addition, this clade of EV-D68 appears to have a specific tropism for lower respiratory tract epithelium.

In this investigation our state of Michigan is certainly on the map. We have had several clusters (8-10 cases) of illness in pediatric patients from a wide variety of geographic regions climbing up to more than 40 children in a single hospital. We hope to get word soon from testing performed on specimens sent to the CDC.

Should your hospital have a potential case that you wish to be tested for EV-D68, here are the steps to be taken:

1) Rule out influenza and RSV. Influenza is just ramping up for the 2014-15 season.
2) If the capability exists, run an enterovirus/rhinovirus PCR. Reports from on-line blogs claim that the xTAG RVP assay will be positive for enterovirus/rhinovirus. Film Array assay when run with EV-D68 may be positive for some combination of rhinovirus primers and negative for the enterovirus primers. ►

► (Cont'd from previous page) This doesn't help you diagnostically as you cannot differentiate the two viruses by this test, but it may be an interesting flag. If the influenza and RSV tests are negative and especially if the EV/RV PCR is positive, consider sending the specimen to the Bureau of Laboratories in Lansing for the CDC to type.

3) Call Epidemiology to get approval for testing. The main number during normal working hours is 517-335-8165. Tell the operator you need to get approval to send an EV-D68 specimen. Collect:
    a) NP Dacron/Plastic swab (optimal specimen) and place in viral transport medium,
    b) 1-2 mL serum (less on a young child) and
    c) ***if*** available on a child with neurologic symptoms, send ½ mL CSF.
    d) Keep all specimens at 4°Cuntil ready to ship.
4) Download a MDCH Microbiology/Virology requisition form and a CDC requisition (CDC test number 10312) both from http://www.michigan.gov/mdch/0,4612,7-132-2945_5103-14806--,00.html for EACH SPECIMEN. These must be filled out electronically
5) You may FAX the forms to 517-335-9871 or email to CDCRecords@michigan.gov.
6) Send the specimens on ice packs overnight to the State Laboratory at address:

    MDCH Bureau of Laboratories
    3350 N. Martin Luther King, Jr. Blvd.
    Lansing, MI 48909

Although EV-D68 is not a reportable infection in the United States, hospitals should report cases and cluster of severe respiratory illnesses to state and local health departments for further guidance and surveillance.

Rare Antimicrobial Resistance Mechanism Detected in Michigan

Carrie Anglewicz, MS
Martha Boehme, MLS(ASCP)CM

The National Healthcare Safety Network (NHSN) newsletter published an article in March 2014 requesting public health laboratories to submit isolates of *Pseudomonas aeruginosa* to the Centers of Disease Control and Prevention (CDC). The CDC is requesting isolates suspected of expressing Verona-Integron-encoded Metallo-β-lactamase (VIM). Isolates of *Pseudomonas aeruginosa* expressing VIM are non-susceptible to penicillins, cephalosporins, cephamycins, and carbapenems. However, they are susceptible to aztreonam. VIM is a carbapenemase, but *P. aeruginosa* is not an *Enterobacteriaceae*, therefore an isolate in this scenario cannot be called►

Carbapenem-Resistant-Enterobacteriaceae (CRE). A recent article published through American Society for Microbiology (ASM) states that the first VIM positive *P. aeruginosa* was identified in 2012 in Ohio. Similar infections were subsequently seen in six other patients in 2012-2013. All cases were found to be linked epidemiologically to the same healthcare facilities and resulted in one death.

Hospitals across Michigan have been submitting *P. aeruginosa* isolates with the applicable resistance pattern to the Michigan Department of Community Health (MDCH) Bureau of Laboratories (BOL) to forward to the CDC since April 2014. Two isolates expressing VIM have been confirmed from southeast Michigan.

At this time, CDC is interested only in isolates of *P. aeruginosa* with the specific resistance pattern mentioned above. However, keep in mind that submission guidelines are updated frequently. MDCH BOL will continue to forward new information to our laboratory partners via listserv. The ASM article can be found at the following link:
http://www.asm.org/index.php/journal-press-releases/93030-highly-drug-resistant-virulent-strain-of-pseudomonas-aeruginosa-arises-in-ohio

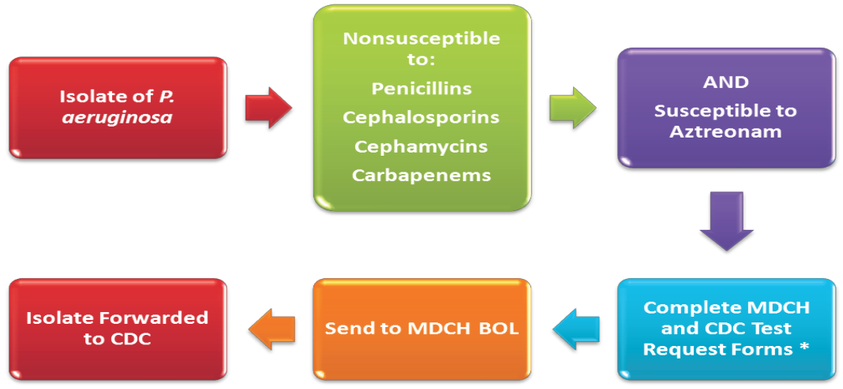

Figure 1. Guidelines for Submitting Isolates if Suspected VIM-expressing *P. aeruginosa.*

*The current MDCH Microbiology/Virology test requisition and CDC specimen submission form are available at:
http://www.michigan.gov/documents/DCH-0583TEST_REQUEST_7587_7.pdf
http://www.michigan.gov/documents/mdch/HUMAN_form-50-34_410210_7.pdf

CDC DASH forms must be filled out electronically (not handwritten). Select test CDC-10223 "Antimicrobial Resistance Testing- Bacterial". In the "Previous Laboratory Results/Comments" box on the second page (near the bottom), free text "suspect VIM". Also attach a printed copy of the antibiotic resistance profile from your facility.

New Developments in the Chemistry and Toxicology Division

Bonita Taffe, Ph.D., M.P.H.
Matthew Geiger, M.S.
Sara Tomechko, Ph.D.

Analytical Chemistry

The Analytical Chemistry Section at the MDCH Bureau of Laboratories is excited to announce the re-establishment of in-house dioxin and dioxin-like compound analysis in fish. Historically, the analysis for these compounds was done by the MDCH BOL; more recently this testing was performed by contract labs.

Dioxin and dioxin-like compounds are among the most toxic chemicals in living organisms, with 2,3,7,8-tetrachlorodibenzo-p-dioxin (2378-TCDD) as the most toxic in this class of compounds. Although environmental levels of dioxins and dioxin-like compounds polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzophenols (PCBs) are declining in the US, exposures in Michigan still exist in areas of known contamination. The Detroit River and the Saginaw Bay watershed area which is downstream from a former source in Midland, Michigan are examples of those areas of known contamination.

EPA and industrial efforts to clean up contamination are progressing; however, sediments in the waterways still contain these compounds, which can result in human exposure through contamination of soils and associated livestock and food sources along the river, especially following riverbed agitation due to flooding. Once ingested, the compounds remain in fat stores and are bioaccumulated in the food chain, which includes sport caught fish consumed by some Michigan residents.

Fish, which are suspected of dioxin exposure, are homogenized and lipids are extracted using a liquid/solid extraction on an ASE300™ accelerated solvent extractor. The lipid weight is calculated, and then the sample is eluted through multiple chromatographic columns to isolate the dioxin and dioxin-like compounds. After concentration, these compounds are analyzed and quantified by gas chromatography-high resolution mass spectrometry (GC-HRMS). With this method, linear calibration curves with R2 values 0.9998 or better over the tested concentration range (0.5 ppb to 2 ppm) with a detection limit of 0.02 ppt are easily obtainable.

►

This method was derived from and is similar to EPA method 1613; in-house modification provide automation and high throughput to the extraction and isolation processes using the ASE300™ accelerated solvent extractor and the J2 Scientific PrepLincTM system. These modifications resulted in cleaner lipid extracts from fish tissue as compared to the contract laboratory that eliminated interferents from the sample, which could result in false positives. A poster entitled "Adaptation of EPA 1613 method to determine concentration of PCDD's and PCDF's by HRGC/HRMS in fish from Michigan waters", Piotr L. Pawlak, Ph.D.; Matthew J. Geiger, MS; Bonita Taffe, Ph.D. MPH, was recently presented by Dr. Pawlak at the 34th International Symposium on Halogenated Persistent Organic Pollutants in Madrid, Spain.

Newborn Screening

The Newborn Screening (NBS) laboratory has been undergoing extensive remodeling and instrumental upgrades this year. Despite working around major construction, the scientific laboratory staff has also been very active in method improvement for the screening program. Several scientists in the NBS laboratory will be presenting platform presentations and posters of their work at the APHL 2014 Newborn Screening and Genetic Testing Symposium (NBSGTS) in Anaheim, CA on October 27-30, 2014. The presentations include:

Seeterlin, M., Stanley, E., Kleyn, M., Hawkins, H., and Taffe, B. (2014, October). *Analysis of False Positive and False Negative MSUD Cases: Using Age Specific Cutoffs to Reduce Both*.

Seeterlin, M., Stanley, E., Hawkins, H., and Taffe, B. (2014, October). *A 5 Minute Extraction Protocol for MSMS: STAT Reporting of Medical Emergency MSMS Profiles.*

TenEyck, K., Wood, H., Foster, L., Muth, E., Patel, A., Burns C., Hawkins, H., and Taffe, B. (2014, October). *Development and Implementation of an Automated DNA Extraction Protocol with Selective Sample Transfer, or 'Cherry Picking', Capabilities for Cystic Fibrosis (CF) Screening*.

Wood, H., Andruszewski, K., Burns C., Hawkins, H., Taffe, B. (2014, October). *A Qualitative Approach For the T cell Receptor Excision Circle (TREC) Assay for the Detection of Primary Immune Deficiency Syndromes (PIDS) Demonstrates Better Sensitivity and Specificity Versus Using a Quantitative Approach.*

Wood, H., Burns C., Hawkins, H., Taffe, B. (2014, October). *A DNA Stability Study for the T cell Receptor Excision Circle (TREC) Real Time PCR Assay Screen for Newborn Immunodeficiency.*

Explore Lab Science– Fishing Event

On August 2nd, the Explore Lab Science Team held an Exploration Day at Hawk Island Park in Lansing, MI. Over fifty students from ages 4 to 17 were in attendance.

Students were able to learn about science topics like DNA, chromatography, and chemical reactions. In addition to science experiments, students participated in a fishing tournament while learning about the Eat Safe Fish Program. We also had a pipetting contest where students were required to pipette colored water into 96-well plates.

Four hands-on experiments were offered throughout the day; 1) Magic Color Breakdown where students learned about chromatography, 2) Mentos Soda Volcano where students learned about surface tension, 3) Melting Ice Rainbows where students learned about states of matter and 4) Banana DNA where students learned how to extract DNA from a banana using common household items.

Trophies and medals were awarded to students that caught the largest fish, the smallest fish and the most fish. In addition, one student was awarded a trophy for our pipetting contest.

The Ingham County Parks donated several gift certificates which were awarded to students during our fishing tournament. Bell's Greek Pizza donated pizza for students and parents. In addition, Meijer donated a gift card that was used to purchase additional fishing supplies and food.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and public health community.

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